Clarification of reconstituted frozen orange juice concentrate by continuous flow centrifugation for limonin glucoside solid phase extraction

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Abstract

BACKGROUND: The suitability of continuous flow centrifugation for the clarification of reconstituted frozen orange juice concentrate prior to its application to a solid phase extraction column for the isolation of limonin glucoside was evaluated. Clarification experiments spanning three flow rates (325, 588 and 875 mL min^{-1}) and three rotor speeds (equivalent to 2130, 8521 and 19 172 × *g*) were conducted in a simple factorial design.

RESULTS: With increasing rotor speed or decreasing flow rate the average particle size, colour parameters (CIE *L, *a**, *b**) and residual solids in the resulting centrifugates were found to decrease, whereas juice characteristics including pH, conductivity, °Brix and limonin glucoside content were unchanged by the clarification process. Mean particle size in the resulting centrifugates ranged from 1.14 to 79.31 μm. The most effective clarification was obtained using a 325 mL min^{-1} flow rate in conjunction with the maximum rotor speed. Suitability of the centrifugates for processing by solid phase extraction was tested through a two-step process, in which the centrifugates were first screened using small columns, followed by application of the centrifugate to a larger self-packed column (6.5 cm × 20 cm) containing SP-70 Sepabeads.**

CONCLUSION: Centrifugates from two conditions (325 and 588 mL min^{-1} at the maximum rotor speed) were suitable for direct application to both columns. It was found that up to 19 L of these centrifugates could be applied to the 6.5 cm × 20 cm column without clogging or experiencing a decrease in flow rate. Analysis of the column effluent revealed that 11 L of centrifugate was sufficient to saturate this column.
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Keywords: frozen orange juice concentrate; clarification; limonoid glucosides; isolation; processing

INTRODUCTION

Limonoids are highly oxygenated triterpenoid compounds that are found in all citrus tissues as limonoid A-ring lactones, glucosides or aglycones (Fig. 1). Limonoid A-ring lactones are water-soluble and tasteless metabolic precursors to limonoid aglycones and glucosides and exist mainly in juice sacs and leaves.[1–5] Limonoid glucosides, also water-soluble and tasteless, are found in especially high concentrations in seed and fruit tissues.[6,7] In contrast, limonoid aglycones are fairly water-insoluble and some, including limonin, the most common aglycone in citrus, have a bitter taste. Aglycones are predominately distributed in leaves and seeds and are sometimes present in juices, but generally only at low concentrations.[8] Juices with aglycone concentrations in excess of 6 mg L^{-1} suffer from poor consumer acceptance owing to their bitterness.[9,10] Those fruits and juices that exhibit time-dependent gradual accumulation of aglycones from their A-ring lactone precursors in excess of this threshold suffer from 'delayed bitterness'. Reports that some citrus limonoids possess biological activities and structural features that influence anti-tumour,[11–15] anti-HIV[16] and triglyceride-lowering properties[17] have resulted in increased interest in these compounds.

Work in our laboratory has focused on the isolation and characterisation of citrus limonoids. Most recently we have undertaken the relatively large-scale isolation of limonoid glucosides in preparation for conducting a study to examine the cholesterol- and triglyceride-lowering properties of limonin glucoside in humans. Limonin glucoside, in addition to having been demonstrated as bioavailable, was chosen over limonin and limonoate A-ring lactone because of the bitterness associated with limonin and the relative ease with which limonoate A-ring lactone is converted

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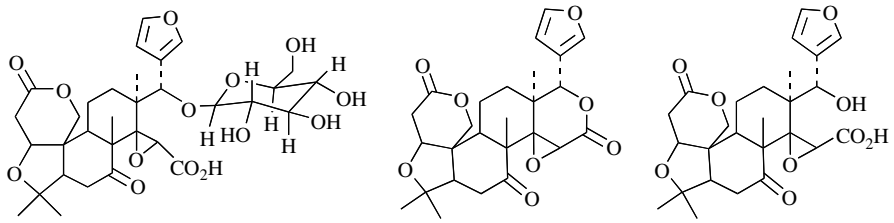

Limonin glucoside
(Limonin 17 β-D-glucopyranoside, LG)

Limonin

Limonoate A-ring lactone

Figure 1. Structures of limonin, limonoate A-ring lactone and limonin glucoside.

to limonin. If large-scale commercial production of a limonin glucoside-containing nutraceutical or functional food is to be undertaken, then an adequate supply of starting material must be identified and an isolation method developed.

The relatively low cost and abundant supply of citrus molasses have resulted in considerable effort being focused on the exploitation of this material as a feedstock for recovering limonoids and other biologically active compounds.[18–20] Most methods for the isolation of limonoid glucosides have thus focused on using citrus molasses as a starting material.[18] Citrus molasses is a conglomerate of waste products, including peel, pulp and rag tissue, from commercial juice production streams that is treated by liming to form a liquid with the consistency of molasses. The limonoid glucoside concentration in citrus molasses is highly dependent on the citrus variety, the harvest time and the molasses liming and subsequent processes, and reported concentrations, normalised to a °Brix of 11.8, have ranged from 209 to 466 mg L^{-1}.[21] In addition, the compositional variation associated with interfering components in the molasses on account of these factors requires that isolation methods go through reoptimisation or be comprehensive.

Orange juice or reconstituted frozen orange juice may offer an attractive alternative as a more consistent and cleaner feedstock for limonoid isolation, because production methods are fairly uniform and juices are not subjected to the liming process which forms additional impurities that may interfere with the isolation process. Wanting to avoid the need to rework an isolation method on a lot-by-lot basis, we sought a more consistent starting material and therefore turned our attention to frozen orange juice concentrate (FOJC).

Each year, over 3.75×10^6 m^3 of FOJC is produced in the USA alone. FOJC does not suffer from the same variation in interfering agents as molasses, since production methods are fairly uniform across producers and FOJC is not subjected to variable harsh treatment processes involved in producing molasses. Any variation in the limonoid glucoside content of sweet orange juice and corresponding FOJC is more likely to be associated with harvest time than any other factor.[3,4,22] FOJC usually contains limonoid glucoside concentrations in the range from 250 to 430 mg L^{-1}, with limonin glucoside accounting for 180 mg L^{-1} of the total glucoside content on average.[23] Most methods for the laboratory-scale isolation of limonoid glucosides focus on a series of chromatographic steps.[18,24–26] However, particulates in the orange juice reduce isolation efficacy. The effects resulting from the presence of particulates can range from slowing of the chromatography to rapid fouling of resins. We explored the use of a continuous flow centrifuge to remove particulates from FOJC as a pretreatment for the juice being subjected to chromatographic isolation. Looking towards the goal that the isolation of limonin glucoside will some day take place on a large-scale level, the objective of this study was to evaluate the clarification of reconstituted FOJC by continuous flow centrifugation on a pilot plant scale and determine the suitability of the resulting centrifugates for direct application to column chromatography isolation.

MATERIALS AND METHODS

Materials

FOJC was obtained from commercial sources and stored at −20 °C. SP-70 Sepabeads were purchased from Supelco (Bellefonte, PA, USA) and Strata-X (30 mg, 1 mL) solid phase extraction (SPE) columns from Phenomenex (Torrance, CA, USA).

Preparation of orange juice

FOJC (18.9 L) was reconstituted in tap water (56.7 L) to 1× strength in a stainless steel container (94.6 L) equipped with a mixing prop. After mixing for 15 min, the liquid was distributed into four containers (18.9 L) and stored in a cold room at 4 °C if not used immediately.

Design of clarification experiments

Reconstituted orange juice (18.9 L) was used for each clarification experiment. Clarification was accomplished using a CARR Powerfuge Pilot centrifuge (Clearwater, FL, USA) with a Masterflex 7520-25 peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) as the feed pump. Juice was introduced into the centrifuge once the desired rotor speed was reached. The flow rate of the feed pump was verified by measuring the quantity of orange juice delivered by the pump over 2 min. Following processing of the 18.9 L of reconstituted juice, the centrifuge was stopped,

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a sample of the clarified juice was taken and the accumulated particulate was collected from the rotor. The centrifuge was cleaned with tap water and hand dried using cheesecloth. Experiments were conducted at three different flow rates (325, 588 and 875 mL min^{-1}) and three different rotor speeds (5 000, 10 000 and 15 000 rpm) in a simple factorial design. The three rotor speeds were equivalent to 2130, 8521 and 19 172 × *g* respectively. The characteristics of the clarified juices were compared with those of an untreated juice sample. Experiments at the lowest rotor speed were conducted in duplicate, while all other experiments were conducted in at least triplicate.

Analysis of reconstituted orange juice

Juice samples were analysed for colour, clarity, soluble solids, conductivity, pH, titratable acids, particle size distribution, limonin glucoside content and % residual solids. For colour analysis, juice samples were placed in a cuvette (20 mm optical path) and the juice colour parameters (CIE L^*, a^*, b^*) were determined using a Minolta CM-508d ChromaMeter (Minolta Camera Ltd, Osaka, Japan). Clarity was determined by measuring the transmittance at 660 nm using the cuvette sample chamber on a Molecular Devices Spectromax 384-Plus plate reader (Sunnyvale, CA, USA). Soluble solids were measured using an Atago PR-100 digital refractometer (Tokyo, Japan) and expressed in °Brix. A YSI model 32 conductance meter (Yellow Springs Instrument Co., Yellow Springs, OH, USA) was used for conductivity measurements. The pH was measured using a Beckmann Instruments model 34 pH meter (Fullerton, CA, USA) standardised to pH 4 and 7. Total titratable acidity was determined with a Brinkmann (Metrohm, Herisau, Switzerland) 682 Titroprocessor used in conjunction with a 665 Dosimat and an E 649 stirrer. The Titroprocessor was calibrated with pH 7 and 10 buffers. The juice sample was diluted 10:1 (v/v) with water, and 100 mL of the test solution was titrated to pH 8.2 using NaOH (0.156 mol L^{-1}). The acid level was calculated as amount of NaOH (mL) × 0.1 and expressed as % citric acid. Particle size distribution was determined using a Horiba LA-900 particle size analyser (Kyoto, Japan). Limonin glucoside content was determined by liquid chromatography/mass spectrometry (LC/MS) as described previously.[7]

For % residual solids determination, samples were centrifuged (30 000 × g, 10 min, 4 °C) using a Sorvall RC 5C Plus floor centrifuge (Dupont Instruments, Doraville, GA, USA). The liquid was discarded and the mass of the pellet was measured. The % moisture content of the pellet was determined using a CEM AVC-80 microwave moisture analyser (Matthews, NC, USA). Approximately 0.5 g of the pellet was placed on the glass fibre pads and dried for 3 min at 100% power. The mass of the pellet was corrected for moisture content and the solid mass per mL of juice was calculated. This ratio (g mL^{-1}) was divided by the solid mass per mL of untreated juice to give the % residual solids value.

Analysis of rotor solids

Following each centrifugation experiment, the total mass of the collected particulate was measured and reported as wet weight. The wet weight was corrected for moisture content by analysing a portion of the solid using a CEM AVC-80 microwave moisture analyser as described above. To ascertain the level of limonin glucoside retained in the solid, 1 g of particulate material was extracted with 10 mL of water. The extract was clarified by centrifugation using a tabletop centrifuge and the limonin glucoside content was determined by LC/MS.[7]

Column loading experiments

Prior to conducting column loading experiments, samples of the clarified juices were applied to Strata-X (30 mg, 1 mL) SPE columns to assess the effectiveness of the clarification conditions. Before applying the sample, the SPE columns were washed with methanol (1 mL) and equilibrated in water (1 mL). The sample, up to 5 mL, was added until the column clogged. For loading experiments, medium from a Biotage 65i flash column cartridge was replaced with SP-70 Sepabeads and the resulting 6.5 cm × 20 cm column was attached to a Biotage Horizon System (Charlottesville, VA, USA). Prior to loading the sample, the column was washed with water (6 L). Clarified juice (3.8–18.9 L) was loaded on the column at a flow rate of 80 mL min^{-1}. After loading the sample, the column was washed with water (6 L) before eluting with 1:1 (v/v) ethanol/water (4 L). During the loading, washing and eluting processes the column effluent was periodically monitored for limonin glucoside content by LC/MS.[7]

RESULTS AND DISCUSSION

FOJC was reconstituted to 1× strength (20.1 ± 0.3 °Brix) and nine different clarification conditions were examined in a simple factorial design consisting of three centrifuge speeds and three flow rates. In Figs 2 and 3 are displayed the wet and dry weight masses respectively of the particulate collected when 18.9 L of orange juice was processed. The mass of the wet material varied from 715 to 1064 g and the moisture content was found to range from 73 to 79%. Dry weight varied from 186 to 282 g. There were no distinct trends within these observations to suggest that one particular condition was better than the others.

In contrast, when the centrifugates were analysed for clarity, % residual solids and colour parameters (CIE L^*, a^*, b^*), the effects of the treatments became apparent. Figure 4 shows the % transmittance measured at 660 nm for the clarified juices relative to 1× strength reconstituted juice. Increases in centrifugal force resulted in a direct improvement in clarity. The effect of flow rate was most discernible when evaluated at the highest centrifuge speed tested,

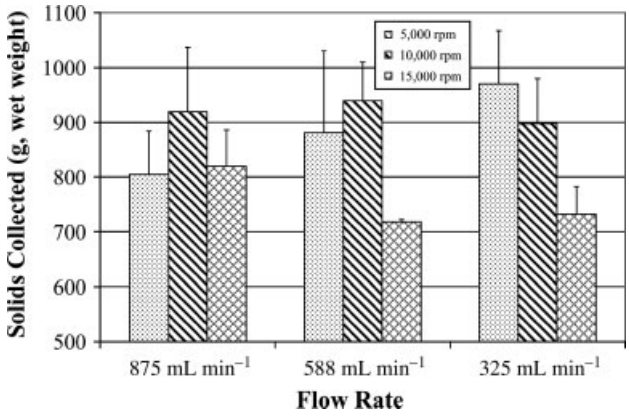

Figure 2. Mass of particulate (g, wet weight) collected when 18.9 L of orange juice was processed.

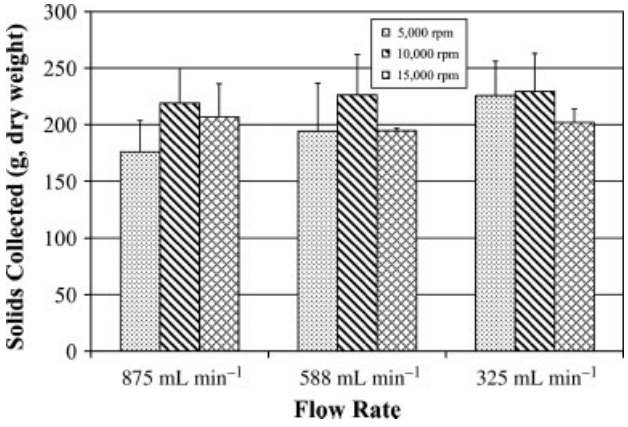

Figure 3. Mass of particulate (g, dry weight) collected when 18.9 L of orange juice was processed.

where decreases in flow rate resulted in improved clarity. Similar results were obtained for % residual solids (Fig. 5) and colour parameters (Table 1).

The decreases observed in turbidity, % residual solids and colour parameters result from the removal of settling pulp and cloud. Cloud is made up of particles that range from 0.4 to 5.0 μm in size and consists of high-molecular-weight proteins, pectins, oil droplets, pigments and membranes. The cloud and settling pulp found in fresh and reconstituted orange juices are important components of the sensory characteristics

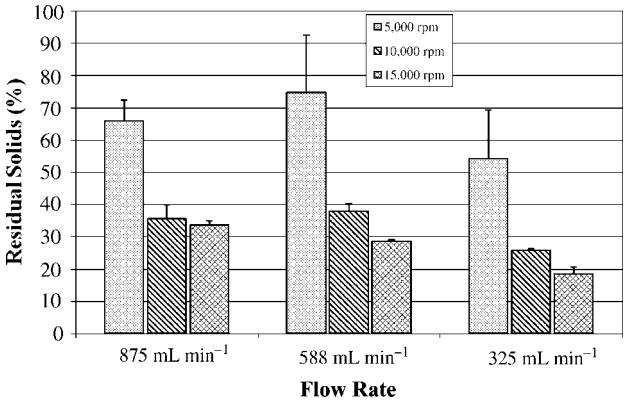

Figure 5. Remaining residual solids following clarification. Percentages are relative to untreated juice. Additional details can be found in the 'Materials and methods' section.

of juices and contribute to the flavour, colour, texture and aroma attributes of these juices. Clarification through natural or mechanical means results in juices with reduced sensory quality. Significant effort has been applied to understanding cloud stability, developing methods for circumventing clarification of citrus juices and producing citrus juice products with improved cloud stability. Settling pulp can be removed with low-speed centrifugation ($1500 \times g$), whereas complete removal of cloud to produce orange juice serum requires high-speed centrifugation or ultracentrifugation,[27] processing by ultrafiltration or treatment with pectinmethylesterase.[28–30] To further characterise the extent of clarification, the mean particle sizes of the juices were measured (Table 1). Going from 5000 to 10 000 rpm for the centrifuge speed resulted in significant decreases in mean particle size. At the highest speed, decreases in flow rate made minor improvements to the clarification. Overall, the best clarification was obtained at a centrifuge speed of 15 000 rpm with a flow rate of 325 mL min^{-1}; under these conditions, over 80% of the solids were removed.

In addition to properties defining the character and quantity of suspended solids in the clarified juices, characteristics associated with soluble juice components, important in planning the downstream chromatography steps, were also measured (Table 1). Differences in sugar/acid ratio, pH and conductivity

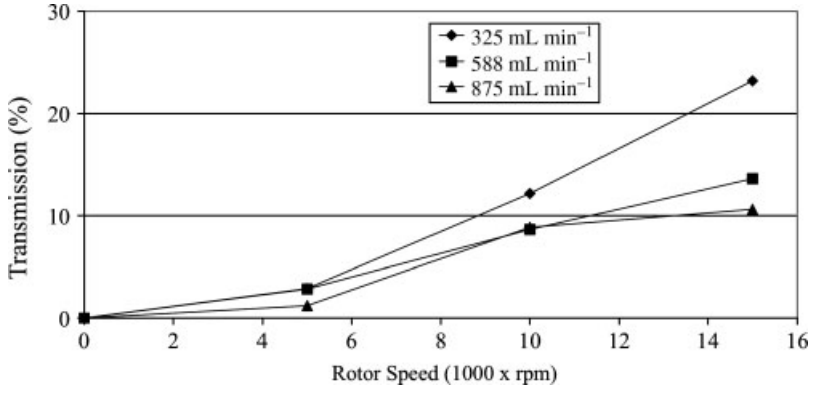

Figure 4. Per cent transmission as a function of centrifugation conditions.

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Table 1. Reconstituted orange juice characteristics for different centrifugation conditions

Rotor speed (rpm)	Flow rate (mL min^{-1})	Sugar/acid ratio	pH	Conductivity (S m^{-1})	Colour			Mean particle size (μm)
					*L**	*a**	*b**	
0	0	19.7	4.0	4.41	42.28	3.95	36.22	236.93
5000	325	19.6	4.1	4.47	40.65	3.00	35.19	79.31
5000	588	19.6	4.1	4.33	40.26	2.89	35.12	63.43
5000	875	19.5	4.1	4.55	41.08	3.21	35.40	65.10
10 000	325	20.0	3.9	4.45	36.64	1.02	30.62	1.45
10 000	588	19.6	3.9	4.49	36.59	1.89	30.37	1.85
10 000	875	19.4	3.9	4.69	37.82	1.52	32.02	1.74
15 000	325	19.6	3.9	4.38	33.27	0.33	25.91	1.14
15 000	588	19.7	3.9	4.33	36.65	0.78	30.33	1.16
15 000	875	19.4	3.9	4.55	37.81	1.34	28.80	1.67

Coefficients of variation were less than 3% for mean particle size measurements and less than 1% for all other measurements.

of reconstituted and clarified juices were found to be negligible. The pre- and post-centrifugation limonin glucoside concentrations of the juices were determined by LC/MS and found not to differ by more than the uncertainty of the LC/MS quantification method itself. The concentration of limonin glucoside was determined to be 180 mg L^{-1}, which is within the range of concentrations previously reported for reconstituted orange juices.[23] Therefore limonoid glucosides, like the other water-soluble components, are unaffected by centrifugation.

A sample of the solid captured from each processing condition was subjected to aqueous extraction and the extract was examined by LC/MS to determine the extent to which limonin glucoside was retained with the solid. For each condition the concentration was found to be at a level that represented less than 1% of the total and we therefore concluded that the extraction of the mass was unwarranted. This result was not unexpected, since limonoid glucosides are water-soluble and, unlike carotenoids, do not readily associate with membranes.

Suitability of the centrifugates for isolation via column chromatography was tested through a two-step process, in which the centrifugates were first screened using small SPE columns, followed by application of the centrifugate to a larger column (6.5 cm × 20 cm). For the small-column experiments, centrifugates obtained from each of the clarification conditions were applied to SPE columns until the column clogged or a total of 5 mL had been applied. Columns to which the centrifugates generated at 5000 rpm were applied immediately clogged. Similar results were obtained for all other columns, with the exception of three to which the centrifugates generated at 15 000 rpm and both 325 and 588 mL min^{-1} flow rates and at 10 000 rpm and 325 mL min^{-1} flow rate were applied. For the large-column experiments the centrifugates generated at 15 000 rpm and both 325 and 588 mL min^{-1} flow rates were tested. We found that up to 18.9 L of centrifugate could be applied without clogging the column or experiencing a decrease in flow rate. Analysis of the column effluent revealed that 11.4 L of centrifugate was sufficient to saturate the column. Past this point, limonin glucoside in any additional centrifugate applied to the column was retained at less than 50% efficiency. Following washing with water to remove sugars and non-retained components, the limonin glucoside was eluted with 1:1 (v/v) ethanol/water, resulting in 95% recovery of the bound limonin glucoside in the first 2 L.

CONCLUSION

Continuous flow centrifugation may be used to generate centrifugates suitable for immediate processing by SPE or column chromatography. For the pilot- or production-scale isolation of limonin glucoside from FOJC, clarification by this method may offer a significant reduction in processing time when compared with the use of standard floor centrifuges. Having a clarification method in hand, continued work is required to determine the economic feasibility of FOJC as a feedstock for the commercial isolation of limonoid glucosides and the benefits that this starting material offers over citrus molasses.

REFERENCES

1 Maier VP and Margileth DA, Limonoic acid a-ring lactone, a new limonin derivative in citrus. *Phytochemistry* **8**:243–248 (1969).

2 Zukas AA, Breksa III AP and Manners GD, Isolation and characterization of limonoate and nomilinoate A-ring lactones. *Phytochemistry* **65**:2705–2709 (2004).

3 Fong CH, Hasegawa S, Coggins CW, Atkin DR and Miyake M, Contents of limonoids and limonin 17-beta-D-glucopyranoside in fruit tissue of Valencia orange during fruit growth and maturation. *J Agric Food Chem* **40**:1178–1181 (1992).

4 Hasegawa S, Ou P, Fong CH, Herman Z, Coggins CW and Atkin DR, Changes in the limonoate A-ring lactone and limonin 17-beta-D-glucopyranoside content of navel oranges during fruit growth and maturation. *J Agric Food Chem* **39**:262–265 (1991).

5 McIntosh CA, Quantification of Limonin and Limonoate A-Ring Monolactone During Growth and Development of Citrus Fruit and Vegetative Tissues by Radioimmunoassay, in *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*, ed. by Berhow MA, Hasegawa S and Manners GD. American Chemical Society, Washington, DC, pp. 73–95 (2000).

6 Hasegawa S, Bennett RD, Herman Z, Fong CH and Ou P, Limonoid glucosides in citrus. *Phytochemistry* **28**:1717–1720 (1989).
7 Schoch TK, Manners GD and Hasegawa S, Analysis of limonoid glucosides from *Citrus* by electrospray ionization liquid chromatography–mass spectrometry. *J Agric Food Chem* **49**:1102–1108 (2001).
8 Hasegawa S, Biochemistry of limonoids in *Citrus*. *ACS Symp Ser* **758**:9–30 (2000).
9 Guadagni DG, Maier VP and Turnbaugh JG, Effect of some citrus juice constituents on taste thresholds for limonin and naringin bitterness. *J Sci Food Agric* **24**:1277–1288 (1973).
10 Guadagni DG, Maier VP and Turnbaugh JG, Effect of subthreshold concentrations of limonin, naringin and sweeteners on bitterness perception. *J Sci Food Agric* **25**:1349–1354 (1974).
11 Lam LKT, Hasegawa S, Bergstrom C, Lam SH and Kenney P, Limonin and nomilin inhibitory effects on chemical-induced tumorigenesis, in *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*, ed. by Berhow MA, Hasegawa S and Manners GD. American Chemical Society, Washington, DC, pp. 185–200 (2000).
12 Tanaka T, Maeda M, Kohno H, Murakami M, Kagami S, Miyake M, *et al*, Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. *Carcinogenesis* **22**:193–198 (2001).
13 Tanaka T, Kohno H, Kawabata K, Honjo S, Miyake M and Wada K, Citrus limonoids obacunone and limonin inhibit the development of a precursor lesion, aberrant crypt foci, for colon cancer in rats, in *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*, ed. by Berhow MA, Hasegawa S and Manners GD. American Chemical Society, Washington, DC, pp. 145–163 (2000).
14 Poulose SM, Harris ED and Patil BS, Citrus limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. *J Nutr* **135**:870–877 (2005).
15 Guthrie N, Morley K, Hasegawa S, Manners GD and Vandenberg T, Inhibition of human breast cancer cells by citrus limonoids, in *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*, ed. by Berhow MA, Hasegawa S and Manners GD. American Chemical Society, Washington, DC, pp. 164–174 (2000).
16 Battinelli L, Mengoni F, Lichtner M, Mazzanti G, Saija A, Mastroianni CM, *et al*, Effect of limonin and lomilin on HIV-1 replication on infected human mononuclear cells. *Planta Med* **69**:910–913 (2003).
17 Kurowska EM, Banh C, Hasegawa S and Manners GD, Regulation of apo B production in HepG2 cells by citrus limonoids. *ACS Symp Ser* **758**:175–184 (2000).
18 Schoch TK, Manners GD and Hasegawa S, Recovery of limonoid glucosides from citrus molasses. *J Food Sci* **67**:3159–3163 (2002).
19 Raman G, Jayaprakasha GK, Brodbelt J, Cho M and Patil BS, Isolation of structurally similar citrus flavonoids by flash chromatography. *Anal Lett* **37**:3005–3016 (2004).
20 Manthey JA, Fractionation of orange peel phenols in ultrafiltered molasses and mass balance studies of their antioxidant levels. *J Agric Food Chem* **52**:7586–7592 (2004).
21 Hasegawa S, Fong CH, Miyake M and Keithly JH, Limonoid glucosides in orange molasses. *J Food Sci* **61**:560–561 (1996).
22 Kita M, Endo T, Shimada T, Moriguchi T, Hirata Y, Hasegawa S, *et al*, Allelic structures of UDP-glucose:limonoid glucosyltransferase affect limonoid bitterness in *Citrus unshiu* and *C sinensis*. *Euphytica* **132**:87–94 (2003).
23 Fong CH, Hasegawa S, Herman Z and Ou P, Limonoid glucosides in commercial citrus juices. *J Food Sci* **54**:1505–1506 (1990).
24 Braddock RJ and Bryan CR, Extraction parameters and capillary electrophoresis analysis of limonin glucoside and phlorin in citrus byproducts. *J Agric Food Chem* **49**:5982–5988 (2001).
25 Tian Q and Ding X, Screening for limonoid glucosides in *Citrus tangerina* (Tanaka) Tseng by high-performance liquid chromatography–electrospray ionization mass spectrometry. *J Chromatogr A* **874**:13–19 (2000).
26 Raman G, Cho M, Brodbelt JS and Patil BS, Isolation and purification of closely related *Citrus* limonoid glucosides by flash chromatography. *Phytochem Anal* **16**:155–160 (2005).
27 Ackerley J and Wicker L, Floc formation and changes in serum soluble cloud components of fresh Valencia orange juice. *J Food Sci* **68**:1169–1174 (2003).
28 Ackerly J, Corredig M and Wicker L, Clarification of citrus juice is influenced by specific activity of thermolabile pectinmethylesterase and inactive PME–pectin complexes. *J Food Sci* **67**:2529–2533 (2002).
29 Corredig M and Wicker L, Juice clarification by thermostable fractions of marsh grapefruit pectinmethylesterase. *J Food Sci* **67**:1668–1671 (2002).
30 Corredig M, Kerr W and Wicker L, Particle size distribution of orange juice cloud after addition of sensitized pectin. *J Agric Food Chem* **49**:2523–2526 (2001).

J Sci Food Agric **88**:2213–2218 (2008)
DOI: 10.1002/jsfa